1 **Remodeling of ryanodine receptor isoform 1 channel** 2 **regulates the sweet and umami perception of *Rattus*** 3 ***norvegicus***

4 Wenli Wang^{1†}, Dingqiang Lu^{2†}, Qiuda Xu², Yulian Jin², Guangchang Gang^{2*}, Yuan
5 Liu^{1*}

6 ¹ Department of Food Science & Technology, School of Agriculture & Biology,
7 Shanghai Jiao Tong University, Shanghai, 200240, China

8 ² College of Biotechnology & Food Science, Tianjin University of Commerce, Tianjin
9 300134, PR China

10 **Abstract:** Sweet and umami are respectively elicited by sweet/umami receptor on the
11 tongue and palate epithelium. However, the molecular machinery allowing to taste
12 reaction remains incompletely understood. Through a phosphoproteomic approach, we
13 found the key proteins that trigger taste mechanisms based on the phosphorylation
14 cascades. Thereinto, ryanodine receptor isoform 1 (RYR1) was further verified by
15 sensor and behaviors assay. A model proposing RYR1-mediated sweet/umami
16 signaling: RYR1 channel which mediates Ca²⁺ release from the endoplasmic reticulum
17 is closed by its dephosphorylation in the bud tissue after umami/sweet treatment. And
18 the alteration of Ca²⁺ content in the cytosol induces a transient membrane
19 depolarization and generates cell current for taste signaling transduction. We

20 demonstrate that RYR1 is a new channel in regulation of sweet/umami signaling
 21 transduction and also propose a “metabolic clock” notion based on sweet/umami
 22 sensing. Our study provides a rich fundamental for a system-level understanding of
 23 taste perception mechanism.

24 **Keywords:** Umami; Sweet; Phosphoproteomic; Ryanodine receptor isoform 1;
 25 Metabolic clock

26 **1. Introduction**

27 Accurate perception of taste information in food is crucial for human and other
 28 animal survival and evaluating food quality. Animals develop a strong preference for
 29 sugar and amino acid/peptide as the fundamental source of energy through TCA cycle
 30 and oxidative phosphorylation. Sweet/umami (sugar and amino acid/peptide) as reward
 31 taste can be elicited by their receptor cells on the tongue and palate epithelium [1]. To
 32 understand taste signal information, many studies focused on the sweet/umami
 33 receptors and the related signaling pathways by live cell imaging, genetics,
 34 bioinformatics and behavioral studies [1-3]. T1Rs, a family of G-protein-coupled
 35 receptors (GPCRs) as the mammalian taste receptors have been identified in sweet and
 36 umami detection [4-6]. Despite relying on different receptors, sweet and umami
 37 transduction use a common signaling pathway and converge on common signaling
 38 molecules, such as transient receptor potential cation channel subfamily M member
 39 (TRPM4 and TRPM5), type 3 inositol-1,4,5-trisphosphate receptors (IP3Rs) and
 40 phospholipase C (PLC β 2), calcium homeostasis modulator 1 (CALHM1) [3, 7-10].
 41 CALHM1 is a voltage-gated ATP-release channel required for sweet and umami taste
 42 perception [10]. IP3Rs are the principal mediator of sweet and umami taste perception
 43 and would link phospholipase PLC β 2 to TRPM5 activation [9]. Moreover, sweet and
 44 umami perception in type II taste receptor cells on the tongue, both relies on the activity
 45 of TRPM4 and TRPM5, which is activated by the change of intracellular calcium
 46 (Ca²⁺) [3, 8, 11, 12]. Together, these studies demonstrated the similarity of sweet and

47 umami in signaling pathway. However, the molecular machines allowing to taste
48 reaction remain incompletely understood. Here, a fundamental question that we should
49 address is whether new receptors/molecules activate TRPM4/TRPM5 and further
50 mediate the signaling transduction of sweet and umami.

51 An intriguing, universal link exists between the Ca^{2+} -mediated signaling and
52 sweet, umami and bitter taste signaling. IP3Rs and ryanodine receptors (RyRs) are the
53 two major calcium (Ca^{2+}) release channels on the sarco/endoplasmic reticulum [13].
54 IP3Rs as the linkers of phospholipase $\text{PLC}\beta 2$ and TRPM5 have been demonstrated in
55 sweet and umami taste perception [9]. Ryanodine receptor isoform 1 (RyR1) is a key
56 mediator of the cellular regulation of calcium homeostasis, modulating multiple
57 intracellular signaling pathways and physiological functions, most importantly in the
58 sarcoplasmic reticulum required for skeletal muscle contract [14]. Ryanodine receptors
59 have verified in the mammalian peripheral Type II and Type III taste receptor cells
60 [15]. RyR1 was selectively associated with L type calcium channels in mammalian
61 taste cells, and they can interact in neurons like in muscle[16]. However, how RyR1
62 mediates sweet and umami signal transduction remains poorly understood.

63 Protein phosphorylation is a mechanism of regulation that is extremely important
64 in complex signal transduction network [17]. Phosphoproteomic as an essential
65 approach is used to understand the complex molecular circuitry of cellular signal
66 transmission [18, 19]. To make a thorough inquiry on the mechanism of taste
67 (sweet/umami) perception, we performed a phosphoproteomic analysis for the rat bud

tissue after 8 typical taste stimuli (sucrose, sucralose, aspartame, thaumatin, succinic acid disodium salt (SUC), monosodium glutamate (MSG), guanosine 5'-monophosphate disodium salt (GMP) and disodium Inosinate (IMP)) treatment (Figure. 1). Before phosphoproteomic analysis, these stimuli concentrations were measured based on taste bud tissue sensor. We discover a role for RYR1 channel in sweet and umami signal transduction, and this is a previously uncharacterized point that regulates taste perception. In addition to providing insights into sweet/umami signal transduction pathways, these data will serve as an important resource for further investigation of nutrient sensing mechanism.

2. Methods

2.1 Material

The 8~10 weeks old Sprague Dawley (SD) rats weighed about 200 g and were all male provided by Shanghai Jiesijie Laboratory Animal Co., Ltd. (Shanghai, China).

2.2 K_a values measurement of 8 taste stimuli based on taste bud tissue sensor

After the SD rats were sacrificed by decapitation, the rat taste bud tissue (from the tip to foliate papillae) was collected and fixed between two nuclear microporous membranes using the sodium alginate-starch gel as a fixing agent as previously described [20]. Due to the gelatinization of sodium alginate solution, this sandwich-type membrane was immersed into 5.0% CaCl_2 solution for 10 s to form the sensing membrane and then fixed on the pretreated glassy carbon electrode. The

88 biosensor with a three-electrode system (a glassy electrode fixed with a taste-measuring
89 membrane as a working electrode, an Ag/AgCl electrode as a reference electrode, and a
90 platinum-wire electrode as a counter electrode) provided a desired microenvironment
91 for ligand-receptor binding.

92 The action potential generated by the taste bud cells after 8 stimuli treatment can
93 be transmitted as electrical signals to the signal acquisition system. The amperometric
94 *i-t* curve method was used to measure the response current of 8 stimuli at a voltage of
95 0.4 V, and ultrapure water as the base fluid was tested. The current change rate was
96 calculated based on the steady state current values at the same time point before and
97 after simulation. Due to substrate saturation effects, the linear concentration range and
98 the kinetic curve of 8 taste compounds were determined, respectively. Their K_a values
99 were calculated according to the method of Wei [21].

100 **2.3 Tissues collection**

101 All rats were done in compliance with the regulations and guidelines of Shanghai
102 Ocean University institutional animal care and conducted according to the AAALAC
103 and the IACUC guidelines. After jejunitis treatment 12 h, taste bud tissue (tongue) was
104 quickly collected and respectively placed in 9% saline solution (including taste stimuli)
105 for 30 min at room temperature, and 9% saline solution was as control in whole
106 experiments. The concentrations of 8 stimuli were defined basing on their K_a values in
107 this study. All samples after treatment were collected for further analysis.

108 **2.4 Protein/peptide preparation**

109 The tissues were cut into pieces and lysed in lysis buffer (100 mM NaCl, 50 mM
110 Tris-HCl pH 7.4, 1% Triton X-100 (v/v) and 1 mM phenyl-methylsulfonyl fluoride).
111 The samples were sonicated with an ultrasound for 100 cycles of 20 min at 4 °C, and
112 then the supernatant was diluted 1:5 with ice-cold acetone and stored at -20 °C for
113 overnight. The pellets were collected by centrifugation again for 10 min at 12,000 g at 4
114 °C. The above two steps were repeated. The total pellets were dried and their protein
115 concentrations were determined using a Bradford assay (Bio-Rad). The proteins were
116 reduced and alkylated in the buffer containing 100 mM triethyl ammonium bicarbonate
117 (TEAB) and 10 mM Tris (2-carboxyethyl) phosphine (TECP) and 20 mM
118 iodoacetamide. Proteins were digested overnight at 37 °C with trypsin (Promega) with
119 an enzyme: substrate ratio of 1:40. and trypsin hydrolysates were labeled using tandem
120 mass tags (TMT) reagent in acetonitrile for 1 h at 25 °C. Samples were dried down and
121 subjected to phosphopeptides analysis.

122 **2.5 Phosphopeptides enrichment and reversed phase nLC-MS/MS analysis**

123 Phosphorylated peptides were enriched using phosphopeptides buffer according to
124 the manufacturer's protocol (TiO₂ beads, GL Sciences, Japan). Phosphorylated
125 peptides were dried down and dissolved in 200 µL of Nano-RPLC buffer A consisting
126 of 2% ACN/0.1% FA. These peptides were subjected to reversed phase LC-MS/MS
127 analysis using Easy-nLC 1000 system coupled to an Orbitrap Q Exactive Plus mass

spectrometer (both Thermo Scientific) for the phosphoproteome analysis. The Nano-RPLC system was equipped with samples a trapping column (ThermoFisher Dionex, PepMap100, C18, 3 μ m, 3 cm $\times$ 100 μ m) and an analytical column (ThermoFisher Dionex, PepMap100, C18, 3 μ m, 15 cm $\times$ 75 μ m). Trapping was performed for 10 min in solvent A at 2 μ L/min. An elution gradient of 5-35% acetonitrile (0.1% formic acid) for 70 mins was used for the phosphoproteome samples analysis. The mass spectrometer was operated via Q Exactive (Thermo Scientific) in data-dependent mode. The Orbitrap Q Exactive Plus full-scan MS spectra from m/z 300–1800 were acquired at a resolution of 70000 for MS and 17500 for MS/MS. Up to 20 most intense precursor ions were selected for fragmentation. HCD fragmentation was performed at normalised collision energy of 25%.

2.6 Data analysis of phosphoproteomics

Raw files were processed using Proteome Discoverer™ (version 1.3) using the SEQUEST® search engine. The data search was performed against the complete rat Swiss-Prot database. Default settings were used, with the following minor changes: methionine oxidation, protein N-term acetylation, protein N-term pyro-glutamate, deamidation of asparagine and glutamine, and methylation of cysteine as variable modifications. TMT isobaric labeling (+229.163 Da) of lysine in protein N-term were set as static modifications. A false discovery rate (FDR) of 1% was applied at the protein, peptide, and modification level.

148 Bioinformatics analysis was performed with Origin 9.1, Microsoft Excel and R
149 statistical computing software. Annotations were downloaded and extracted from
150 UniProtKB, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes
151 (KEGG). In addition, protein-protein interaction networks were obtained by STRING
152 database (<http://string.embl.de/>). All figures were finally organized in Adobe Illustrator
153 CS6.

154 **2.7 The role of RYR1 based on taste bud tissue sensor**

155 According to the previous preparation method of sensor[20, 22], the rat taste bud
156 tissue from SD rat was collected. Using the sodium alginate-starch gel as a fixing agent,
157 taste bud tissues were fixed between two nuclear microporous membranes, and they
158 were made into a sandwich structure. This prepared membrane structure was immersed
159 in 5 g/100 mL CaCl_2 solution for 10 s to fix them well. Then physiological saline was
160 used to remove remaining Cl^- , Ca^{2+} on the membrane. The fixed membrane structure
161 containing taste bud tissue was respectively placed in the ryanodine solution with
162 different concentrations (10^{-5} ~ 10^{-6} mol/L) for 30 min and then fixed on the surface of
163 glassy carbon electrode mentioned above. This sensor was determined by
164 amperometric *i-t* curve method in a three-electrode system, and then the response
165 current was plotted for a series of MSG solution (10^{-6} ~ 10^{-12} mol/L). The rate of current
166 change as detection index was used to calculate the role of RYR1 in the response of
167 MSG to taste bud tissue.

168 **2.8 Taste behavioral assays**

169 Taste behavior was assayed using a short-term assay that directly measures taste
170 preferences by counting water intake volume. On testing days, each three rats were
171 placed into the cage, and presented with water and two different tastant solutions (300
172 mmol/L sucrose and 50 mmol/L MSG+1mmol/L IMP) [23]. Each 500 μ L 1×10^{-5} mol/L
173 ryanodine solution was smeared in the tongue of rat and 2 $\times$ per day. The rats without
174 ryanodine solution as the control groups were investigated in comparison to the treated
175 groups. Water consumption was measured in each over 24 h. After an initial recording
176 period of 24 h, the samples were renewed and the locations were switched to control for
177 side bias. Water consumption was then measured over another 24 h. Tubes were of
178 identical construction and all individual drinking tubes were tested over night before
179 the experiment to ensure that they did not lose water through dripping.

180 **2.9 Immunostaining of RYR1 in taste bud tissue**

181 Rat taste bud tissues were fixed with a 4% paraformaldehyde solution.
182 Paraffin-embedded tissues with 3 μ m thick sections were deparaffinized with xylene
183 for 3 $\times$ 30 minutes and incubated in graded concentration of ethanol and then washed
184 with PBS for 3 $\times$ 5 minutes. The dewaxed and rehydrated sections were stained in
185 hematoxylin solution until the nuclei becoming blue. The sections were washed twice
186 with 70% ethanol, absolute ethanol and xylene for 10 min, respectively. The dewaxed
187 and rehydrated samples of paraffin section were incubated in boiling 1 mM EDTA

188 buffer (pH=8.0) for 2 min, and sections were washed with PBS with 0.1% Triton
189 X-100 (PBST), blocked with 3% goat serum (Invitrogen, US) in PBST. Sections were
190 incubated with primary antibody (rabbit anti-RYR1 antibody 1:500, AB9078,
191 millipore) overnight at 4 °C, incubated with fluorescence-tagged secondary antibody
192 (goat anti-rabbit IgG 1:1000, AB6721, USA) for 30 min at room temperature. The
193 sections were examined using an Olympus light microscope (CX31, Olympus).

194 **2.10 Double-label immunofluorescence for RYR1 and calnexin in bud tissue**

195 The fixed tissues were placed in 30% sucrose solution overnight at 4 °C for
196 cryoprotection. Tissues were embedded in OCT compound and sectioned at 5 µm
197 thickness on a cryostat. Sections were washed in PBS with 0.1% Triton X-100 (PBST),
198 blocked with 10% donkey serum in PBST, incubated with primary antibodies (RYR1,
199 1:400, abcam2868, US and calnexin, 1:500, abcam22595, US) overnight at 4 °C, and
200 incubated with the Alexa Fluor 488-conjugated donkey anti-mouse IgG (1:1000, abcam
201 150105, US) and Alexa Fluor 594-conjugated donkey anti-rabbit IgG (1:1000, abcam
202 150076, US) as the secondary antibodies for the RYR1 and calnexin antibodies,
203 respectively. The sections were observed under a confocal laser scanning microscope
204 (FV1200, Olympus, Japan). The Green fluorescence was excited with 488 nm and
205 imaged through a 525nm long-path filter. The Red fluorescence was excited at 543 nm
206 and imaged through a 590 nm long-path filter.

207 **2.11 Statistical analysis**

208 To ensure the consistency and reproducibility of our results, we conducted at least
209 three repeated trials performing in different preparations and from at least three
210 different animals.

211 **3. Results**

212 **3.1 The allosteric constant of 8 taste stimuli for rat bud tissue**

213 In consideration of the effect of taste stimuli concentration on protein
214 phosphorylation, we measured the response ranges of diverse sweet and umami
215 substances including protein, nucleotide, amino acids and sugar by rat bud tissue
216 sensor. This sensor maintained the activity and integrality of taste bud cells so that the
217 information of ligand-taste receptor binding can be well collected by electrical signals.
218 The allosteric constants (K_a) of ligand-receptor, which were defined as the
219 concentration of ligand at half of the maximum output signals of cells, were calculated
220 by the signal output generated by the interaction of the receptors in bud tissue to ligands
221 (sweet or umami molecules)[20]. We found that this sensor provided the response
222 signals for 8 taste stimuli at low concentrations (10^{-13} mol/L). The K_a values of 8 taste
223 stimuli were listed in Supplementary table 1. These results implied that sweet/umami
224 receptors in rat bud tissue had the excellent recognition and sensing effect to 8 taste
225 stimuli.

226 The perception ability of four umami substances in rat taste bud tissue is:
227 GMP>IMP>SUC>MSG. However, the sensing ability of umami hT1R1 towards the

228 abovementioned four umami ligands was much lower than bud tissue[24]. This may be
229 related to umami taste transduction mechanism based on multiple umami receptor
230 (heterodimers T1R1/T1R3 and metabotropic glutamate receptors)[7] or the cross-talk
231 among various signal pathways in bud cell[8, 11]. Artificial sweeteners can activate the
232 same sweet taste receptor on the tongue, e.g., aspartame as artificial sweetener showed
233 a certain sensing performance of sweet taste receptor nanovesicles-based bioelectronics
234 tongues[25]. In this study, the perception ability of four sweet stimuli is:
235 Sucrose>Aspartame>Sucralose>Thaumatococcus (Supplementary table 1). Together, to gain
236 insight into sweet and umami taste signal networks whose activity is regulated by
237 protein phosphorylation, we used the K_a values of 8 taste stimuli to respectively treat rat
238 bud tissues for 30 min and collected these tissues for phosphoproteomic analysis.

239 **3.2 Analysis of the differential phosphoproteins of stimulated taste bud tissue**

240 To identify the relevant effectors involved in sweet and umami signal
241 transduction, we used a proteomic approach to identify phosphoproteins and their
242 phosphorylation sites in the stimulated taste bud tissue. Phosphoproteins were analyzed
243 and identified by liquid chromatography tandem mass spectrometry (LC-MS/MS)
244 combined with a *Rattus norvegicus* Uniprot database. Protein and peptide false
245 discovery rates were set at 1%. We defined the identified phosphoproteins as
246 differential proteins if there was a fold change in excess of 1.2 or in less of 0.83 proteins
247 changed significantly in the stimulated taste bud tissues compared with control. For
248 four sweet stimuli, we identified the total 196 differential proteins, which clearly

revealed that substantial changes in the phosphorylation levels (Figure. 2a). Of these, 125 phosphoproteins (78 upregulated and 47 downregulated) were identified in the bud tissue of thaumatin treatment, containing 470 phosphorylation sites. More than 60% phosphoproteins contained more than one phosphorylation site, with some proteins having as many as 27 sites. Among other sweet molecules, aspartame showed 98 phosphoproteins (53 upregulated and 45 downregulated), whereas data from of sucrose and sucralose showed 61 and 38 phosphoproteins, respectively. 19 differential phosphoproteins as the shared ones were accounted in the bud tissues from four sweet stimuli by Venn analysis (Figure. 2b). Phosphorylation in the shared proteins was biased toward Ser compared Thr (76%: 17%), a similar with the findings in eukaryotes, where Ser phosphorylation may account for 80-90% of total phosphorylation sites[26].

Though there were many differential phosphoproteins in the bud tissue of thaumatin and aspartame stimuli, we still identified more differential phosphoproteins in the bud tissues from umami stimuli than sweet molecules. A total of 239 differential phosphoproteins were identified in the bud tissues from four umami stimuli compared with the unstimulated bud tissues (Figure. 2a). Of these, 143 differential phosphoproteins (61 upregulated and 82 downregulated) in the bud tissue of GMP treatment account for 60% of total differential phosphoproteins, containing 551 phosphorylation sites. Next, we examined 94 and 71 differential proteins in the bud tissue of MSG and IMP treatment, respectively. In contrast, the minimal amounts of differential phosphoproteins were identified in the bud tissue of SUC treatment. Closer

inspection of these data, we found that protein phosphorylation of taste bud tissue would be easier in present of taste stimuli including amino-group. These data suggested an interesting insight regarding the 9 shared protein from four umami stimuli, and 8 downregulated phosphoproteins also were found in the bud tissues from four sweet stimuli as the shared ones (Figure. 2c&d).

Based on the studies of the Ca^{2+} -mediated sweet (umami) taste signaling[5, 6, 27], the related differential phosphoproteins to Ca^{2+} channel should be summarized to elucidate sweet and umami signaling transduction (Table S4). The 8 taste stimuli were shown by a number of specific related phosphoproteins in the corresponding protein levels (Figure. 3). Several key proteins in Ca^{2+} -mediated signaling were found in those taste bud tissues after sweet and umami stimulation, such as histidine-rich calcium binding protein (HRC), RYR1 and ATPases (Figure. 2e). It is particularly noteworthy that, RYR1 as a shared differential phosphoprotein was found in all the treated taste bud tissues.

3.3 A series of key protein related to Ca^{2+} -mediated sweet (umami) taste signaling

As mentioned above, these key proteins that may mediate the connectivity of umami/sweet signaling transductions, were further analyzed according to KEGG pathway analysis and STRING database (<http://string.embl.de/>) (Figure. 2e&f). Closer inspection of these data, however, provides interesting insights regarding the regulation of sweet and umami taste pathway by calcium signaling. RYR1, which mediates the

290 release of from intracellular stores into the cytosol[14], is a shared differential
 291 phosphoprotein in all bud tissues after sweet and umami stimuli. HRC that can directly
 292 interact with sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) to regulate Ca^{2+}
 293 homeostasis including the regulation of both Ca^{2+} -uptake and Ca^{2+} -release[28], also
 294 found in the mostly bud tissues after sweet and umami stimuli. Homer proteins regulate
 295 a number of Ca^{2+} handling proteins, including transient receptor potential channels and
 296 other Ca^{2+} permeable channels, ionotropic and metabotropic glutamate receptor, shank
 297 scaffolding proteins or endoplasmic reticulum (ER) Ca^{2+} channels[29]. Homer3 as an
 298 important differential phosphoprotein were found mostly (6/8) bud tissues samples
 299 after 8 taste stimuli treatment. Srl involved store-operated calcium entry (Gene
 300 Ontology: 0002115), is also a shared differential phosphoprotein.

301 In the perception of sweet and umami, despite relying on different receptors, their
 302 transductions converge on common signaling molecules[8], among which calcium
 303 homeostasis modulator 1 is expressed specifically in sweet/umami-sensing type II taste
 304 bud cell [10]. Thaumatin as a sweet protein activated calcium-sensing receptor with an
 305 EC_{50} value of 71 μM [30]. The previous studies demonstrated the similarity of sweet
 306 and umami in perception process which relies on the activity of TRPM5 and the
 307 relevant Ca^{2+} signaling molecules, such as type 3 inositol-1,4,5-trisphosphate receptor,
 308 phospholipase C and calcium homeostasis modulator 1 [7-10]. The shared proteins
 309 RYR1 as an intracellular Ca^{2+} release channel has a functional coupling with IP3R in
 310 regulating intracellular Ca^{2+} signaling in skeletal muscle[31]. Nevertheless, IP3R is a

311 common signaling molecule in sweet and umami transduction signaling pathway[9]. In
312 current study, a series of key protein related to Ca^{2+} -mediated signaling demonstrated a
313 critical role of RYR1 in controlling sweet (umami) taste perception.

314 **3.4 The roles of RYR1 in MSG perception based on rat taste bud tissue sensor**

315 We reasoned that RYR1 as a key channel regulates Ca^{2+} signaling after sweet
316 (umami) stimuli. Based on previous study, electrochemical signal generated by the
317 interconnect allosteric cascade between the receptor and its ligand can truly reflect the
318 biological effects of cell signaling amplification and transmission[20, 22, 24]. To prove
319 the role of RYR1 in sweet (umami) perception, we further assembled a series of rat
320 taste bud tissues with ryanodine at the concentrations of 10^{-5} ~ 10^{-6} mol/L and
321 constructed their tissue sensors. The changing rate of response current showed a
322 decreasing relationship with the concentration of ryanodine comparison to the
323 biosensor without assembled ryanodine (Figure. 4a). Increasing the concentration of
324 ryanodine tended to decrease the response strength of MSG on sensor. As predicted, the
325 ryanodine as an inhibitor of rat taste bud tissue has been interpreted when the
326 concentration of MSG was at 10^{-12} ~ 10^{-6} mol/L. The response of MSG to taste bud tissue
327 was fully reduced in the presence of 4×10^{-6} mol/L ryanodine. Our result indicated that
328 ryanodine inhibited the activity of RYR1 and further slowed down umami signaling.
329 This finding suggested that RYR1 was required to keep umami signaling pathway
330 integrality.

331 **3.5 The effective of RYR1 on behavioral tests**

332 To further investigate the influence of RYR1 on taste perception, rats were
333 exposed to different chemical stimuli (sucrose, IMP and MSG) to monitor their
334 behavior. Rats, which was smeared in the tongue with 1×10^{-5} mol/L ryanodine solution,
335 were presented with water, 300 mM sucrose and 50 mM MSG+1 mM IMP, the
336 unsmeared rats as wild-type control group. Consistent with the sensor results, we
337 examined the water intake volume on umami solution for the rats after ryanodine
338 treatment has a decreasing trend than control ones (Figure. 4b). However, there is an
339 interesting result that the water intake volume on sucrose solution for the rats after
340 ryanodine treatment showed an increasing. In order to explain this reason and avoid the
341 effect of chemical concentration on the water intake volume, we performed the
342 experiment at a lower concentration (sucrose 1×10^{-7} mol/L, IMP 1×10^{-8} mol/L+ MSG
343 5×10^{-7}) based on sensory pretest. The simulation results showed the total water intake
344 volume for the rats after ryanodine treatment has a decreasing trend than control ones.
345 Our results showed that sensitivity (the water intake volume) to sweet and umami was
346 markedly inhibited in rats with ryanodine treatment. The results described above
347 validated RYR1 as a key mediator is important in influencing taste perception for both
348 sweet and umami.

349 **3.6 Expression profiling of RYR1 and the related protein in the tongue**

350 Ryanodine receptors (RyRs) with extraordinary size and complexity locate in the
 351 sarco/endoplasmic reticula (SR/ER) of most metazoan cell types[14]. The previous
 352 studies showed RYR1 required for skeletal muscle contraction is the predominant
 353 isoform[14]. Though calcium homeostasis modulators (CALHMs) are abundantly
 354 expressed in taste bud cells and have an important role in sensing sweet, bitter and
 355 umami[10]. Considering the result of RYR1 in the present manuscript, it is likely
 356 needed to observe the distribution of RYR1 in rat taste bud tissue. The
 357 immunohistochemical assay confirmed robust activation of RYR1 in rat bud tissue.
 358 (Figure. 4c), suggesting a mechanism for RYR1 channel gating by Ca^{2+} , is essential for
 359 numerous cellular functions including sweet/umami signaling transduction in taste bud
 360 tissue.

361 Sweet and umami substances, which both provide sugar and amino acid/peptides,
 362 are the fundamental sources of energy for animals. Several studies have implicated a
 363 role for calnexin in controlling mitochondrial metabolism by Ca^{2+} flux, thereby
 364 regulating the energetic output of the oxidative phosphorylation pathway^[32, 33]. To
 365 further investigate a potential role of calnexin for RYR1 mediated taste signaling, we
 366 also confirmed a predominant co-location of RYR1 with calnexin in the rat bud tissue
 367 and the tongue muscle via confocal microscopy (Figure. 4e). An example is the
 368 abundant calnexin, which stimulated the ATPase activity of sarco/endoplasmic
 369 reticulum Ca^{2+} ATPase (SERCA) [32]. Combined with the results of phosphoproteome,
 370 the two proteins most significantly associated with ATPase activity of SERCA. This

371 finding suggested that calnexin was required in taste bud tissue to keep the activity of
 372 SERCA and regulate voltage-gated Ca^{2+} channels for sweet and umami perception.
 373 These findings together strongly support a notion that RYR1 related to Ca^{2+} signaling
 374 transduction proteins play a role in controlling Ca^{2+} flux in taste bud tissue following
 375 sweet and umami-tasting compound ingestion.

376 **4. Discussion**

377 Based on multiple signaling pathways, potential taste chemicals are precepted by
 378 interacting with taste receptor cells. Two distinct salt transduction mechanisms which
 379 are the epithelial sodium channel ENaC identified as the underlying receptor for
 380 mediating low-salt attraction and the amiloride-insensitive high salt transduction in
 381 Type II TRCs dependent on the presence of the Cl^- anion, have been reported [34, 35].
 382 Recent study showed that a subset of excitatory neurons in the pre-locus coeruleus
 383 express prodynorphin, and these neurons are a critical neural substrate for
 384 sodium-intake behavior [36]. Acids are detected by type III taste receptor cells (TRCs),
 385 and the Otop1 expressed in type III TRCs has been proposed as a candidate sour
 386 receptor[37]. Bitter, sweet and umami stimuli are usually detected by the type II cells
 387 which express taste receptor type 1 (T1R) [5, 6] or T2R [38]. In addition, broadly
 388 responsive taste cell, as a subset of type III cells respond to sour stimuli but also use a
 389 $\text{PLC}\beta$ signaling pathway to respond to bitter, sweet and umami stimuli [39]. Calcium
 390 homeostasis modulator 1 (CALHM1) is expressed specifically in type II taste bud cells,
 391 which is a voltage-gated ATP-release channel required for sweet, umami and bitter

392 taste perception [10]. Their transductions converge on the common signaling proteins
393 (likes PLC β 2, IP $_3$ and TRPM4/TRPM5 selectively expressed in taste tissue), despite
394 relying on different receptors [8].

395 Sweet (sugar) and umami (amino acid/peptides) compounds provide energy (ATP,
396 GTP and carbon) for animals via oxidative phosphorylation, TCA cycle and glycolysis.
397 Sweet and umami stimuli are detected in the same cells [40] and share a common
398 subunit T1R3 [4]. In spite of their diversity, T1Rs converge on a common intracellular
399 signaling pathway, and their transporters couple to Ca $^{2+}$ signaling. Sweet and umami
400 stimuli interact with receptors to generate second messengers, or taste stimuli itself is
401 transported into the cytoplasm of taste bud cell and activates downstream events[41].
402 When T1Rs are activated by sweet or umami stimuli, G $\beta\gamma$ dimers (belong to the family
403 of GPCRs) are released, which stimulates PLC β 2 to mobilize intracellular Ca $^{2+}$. The
404 elevated Ca $^{2+}$ levels in the cytosol lead to the opening of TRPM5 that effectively
405 depolarizes taste cell. In this study, the key differential protein and their possible roles
406 in taste are discussed below.

407 We found a shared phosphoprotein RYR1, which is a well-known family of
408 ligand-gated channels responsible for the release of Ca $^{2+}$ from the intracellular Ca $^{2+}$
409 stores. A number of proteins-calmodulin, homer proteins and RYR1 stabilizing subunit
410 calstabin1 (FKBP12)-bind and regulate RYR1 open-closed structure [42-44]. The
411 binding of FKBP12 to RYR1 promoted and stabilized the closed state of the RYR1
412 calcium channel required for excitation-contraction coupling in skeletal muscle, but

413 FKBP12-depleted, PKA-hyperphosphorylated, S-nitrosylation of RYR1 resulted in
 414 Ca^{2+} leak via the opening of RYR1 channels[44]. In this study, RYR1 containing S2840
 415 and T4464 sites is progressively dephosphorylated in all treated taste bud tissues
 416 (Table. S3). Moreover, the RYR1 related proteins show a significant down change,
 417 with the downregulation of several kinases and the upregulation of phosphatase (Table.
 418 S4). We infer that the RYR1 channel exhibited weak “leaky” channel behavior (open
 419 state) when animals get hungry; during this time, Ca^{2+} slowly releases into the cytosol
 420 via RYR1 channel. However, the RYR1 channel in bud tissue would be closed after
 421 sweet and umami substances ingestion (Figure. 5). The alteration of Ca^{2+} content in the
 422 cytosol can induce a transient membrane depolarization. TRPM5, which responds to
 423 the rate of change in Ca^{2+} concentration, is directly activated at a certain range to
 424 generate significant cell current for taste signal transduction[12].

425 Histidine-rich Ca-binding protein (HRC) as the key differential protein also was
 426 found in the present study. HRC as the regulator of SERCA2a and RYR2 mediates SR
 427 Ca^{2+} homeostasis[28]. Moreover, the phosphorylation of HRC on Ser96 by Fam20C
 428 regulated the interactions of HRC with SERCA2a, suggesting a new mechanism for
 429 regulation of SR Ca^{2+} homeostasis[45]. In this study, we found the HRC containing 25
 430 phosphorylation sites was robustly dephosphorylated, suggesting its role in regulating
 431 Ca^{2+} cycling that is critical for sweet and umami signaling. The above clusters also
 432 recruit other proteins implicated in the regulation of Ca^{2+} signaling, for example, the
 433 sarcoendoplasmic reticulum Ca^{2+} -ATPase (SERCA) is identified as a differential

434 protein. The activity of SERCA was stimulated by chaperone calnexin, which increased
435 the ER Ca^{2+} content and controls mitochondrial Ca^{2+} responses[32]. Thus, the calnexin
436 in rat bud tissue probably control the flux of Ca^{2+} in taste signaling by regulating the
437 activity of SERCA. In addition, the shared differential protein sarcalumenin (SAR), a
438 Ca^{2+} -binding glycoprotein located in the longitudinal SR, regulates Ca^{2+} reuptake by
439 interacting with SERCA[46]. The study has demonstrated SAR is essential for
440 maintaining the Ca^{2+} transport activity of SERCA2a[46].

441 Changes in Ca^{2+} content in the cytosol via the regulation of RYR1 may due to the
442 related protein amounts or activities. Homers are scaffolding proteins that bind GPCRs,
443 IP3 receptors, RYR1, PLC β and TRP channels through the CC region and selection of
444 specific polyproline-rich sequences (PPXF) [29, 47]. Homer that cross-link G
445 protein-coupled metabotropic glutamate receptor (mGluRs) is enriched at the
446 postsynaptic density [48]. Homer3 was phosphorylated by CaMKII and activated by
447 glutamate stimuli, resulting in attenuation of the physical and functional coupling
448 between mGluR1 and IP3R [49]. Moreover, activity-induced Homer proteins further
449 dynamically regulate the communication between calcium signaling related proteins to
450 achieve the facilitation of calcium currents in neurons[50]. Homer3 as a key differential
451 protein also was found based on the result of phosphorproteome, so its phosphorylation
452 state regulates the physical coupling between mGluR1 and IP3R1 which partly reflects
453 the Ca^{2+} signal during sweet and umami signaling pathway. Our data do not exclude the

possibility of other Ca^{2+} -dependent pathway by the induction by sweet and umami stimuli.

5. Conclusion

In summary, we have identified the key proteins in the rat bud tissue after sweet and umami stimuli by a phosphoproteomic approach. Our findings reveal a molecular mechanism in sweet and umami perception by a series of RYR1 related phosphoproteins. For the taste bud tissue treated by sweet and umami substances, SERCA pump is activated by the regulation of dephosphorylated HRC and SAR to increase Ca^{2+} flux from the cytosol to the ER, and the close of RYR1 channel by dephosphorylation to stop the ER Ca^{2+} leak. Thus, the concentration of Ca^{2+} in the cytosol is significantly decreased. The alteration of Ca^{2+} content induces a transient membrane depolarization and generates cell current for sweet and umami signaling transduction. RYR1 channel in bud tissue as a new one in regulation of sweet and umami signaling transduction is verified in this study. Additionally, a “metabolic clock” notion based on nutrient (sweet and umami) sensing mechanism was proposed.

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Author contributions

473 Wenli Wang performed experiments, analysed data, wrote the paper. Dingqiang
474 Lu performed experiments, analysed data. Qiuda Xu and Yulian Jin performed
475 experiments. Guangchang Gang conceived the project, designed experiments, analysed
476 data, provided funding. Yuan Liu conceived and supervised the project, provided
477 funding.

478 **Competing interests**

479 The authors declare no competing interests.

480 **Appendix A. Supplementary data**

481 **Supplementary data (Table S1)** to this article can be found online at

482

483 **References**

- 484 [1] G.Q. Zhao, Y.F. Zhang, M.A. Hoon, J. Chandrashekar, I. Erlenbach, N.J.P. Ryba, C.S. Zuker, The
485 receptors for mammalian sweet and umami taste, *Cell* 115(3) (2003) 255-266.
- 486 [2] B. Kalyanasundar, G.D. Blonde, A.C. Spector, S.P. Travers, Electrophysiological responses to sugars
487 and amino acids in the nucleus of the solitary tract of type 1 taste receptor double-knockout mice, *J*
488 *Neurophysiol* 123(2) (2020) 843-859.
- 489 [3] D. Dutta Banik, L.E. Martin, M. Freichel, A.-M. Torregrossa, K.F. Medler, TRPM4 and TRPM5 are
490 both required for normal signaling in taste receptor cells, *Proceedings of the National Academy of*
491 *Sciences* 115(4) (2018) E772-E781.
- 492 [4] X.D. Li, L. Staszewski, H. Xu, K. Durick, M. Zoller, E. Adler, Human receptors for sweet and umami
493 taste, *Proceedings of the National Academy of Sciences of the United States of America* 99(7) (2002)
494 4692-4696.
- 495 [5] G. Nelson, J. Chandrashekar, M.A. Hoon, L.X. Feng, G. Zhao, N.J.P. Ryba, C.S. Zuker, An amino-acid
496 taste receptor, *Nature* 416(6877) (2002) 199-202.
- 497 [6] G. Nelson, M.A. Hoon, J. Chandrashekar, Y.F. Zhang, N.J.P. Ryba, C.S. Zuker, Mammalian sweet
498 taste receptors, *Cell* 106(3) (2001) 381-390.
- 499 [7] S.C. Kinnamon, Umami taste transduction mechanisms, *Am J Clin Nutr* 90(3) (2009) 753s-755s.
- 500 [8] Y.F. Zhang, M.A. Hoon, J. Chandrashekar, K.L. Mueller, B. Cook, D.Q. Wu, C.S. Zuker, N.J.P. Ryba,
501 Coding of sweet, bitter, and umami tastes: Different receptor cells sharing similar signaling pathways,
502 *Cell* 112(3) (2003) 293-301.
- 503 [9] C. Hisatsune, K. Yasumatsu, H. Takahashi-Iwanaga, N. Ogawa, Y. Kuroda, R. Yoshida, Y. Ninomiya,
504 K. Mikoshiba, Abnormal taste perception in mice lacking the type 3 inositol 1,4,5-trisphosphate
505 receptor, *J Biol Chem* 282(51) (2007) 37225-37231.
- 506 [10] A. Taruno, V. Vingtdeux, M. Ohmoto, Z.M. Ma, G. Dvoryanchikov, A. Li, L. Adrien, H.T. Zhao, S.
507 Leung, M. Abernethy, J. Koppel, P. Davies, M.M. Civan, N. Chaudhari, I. Matsumoto, G. Hellekant,
508 M.G. Tordoff, P. Marambaud, J.K. Foskett, CALHM1 ion channel mediates purinergic
509 neurotransmission of sweet, bitter and umami tastes, *Nature* 495(7440) (2013) 223-226.
- 510 [11] K. Yasumatsu, Y. Ogiwara, S. Takai, R. Yoshida, K. Iwatsuki, K. Torii, R.F. Margolskee, Y. Ninomiya,
511 Umami taste in mice uses multiple receptors and transduction pathways, *J Physiol-London* 590(5)
512 (2012) 1155-1170.

513 [12] D. Prawitt, M.K. Monteilh-Zoller, L. Brixel, C. Spangenberg, B. Zabel, A. Fleig, R. Penner, TRPM5 is
514 a transient Ca²⁺-activated cation channel responding to rapid changes in [Ca²⁺]_i, *Proceedings of the*
515 *National Academy of Sciences of the United States of America* 100(25) (2003) 15166-15171.

516 [13] M.-D. Seo, M. Enomoto, N. Ishiyama, P.B. Stathopoulos, M. Ikura, Structural insights into
517 endoplasmic reticulum stored calcium regulation by inositol 1,4,5-trisphosphate and ryanodine
518 receptors, *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1853(9) (2015) 1980-1991.

519 [14] A. des Georges, O.B. Clarke, R. Zalk, Q. Yuan, K.J. Condon, R.A. Grassucci, W.A. Hendrickson, A.R.
520 Marks, J. Frank, Structural Basis for Gating and Activation of RyR1, *Cell* 167(1) (2016) 145-+.

521 [15] M.A. Rebello MR, Medler KF, Ryanodine Receptors Selectively Interact with L Type Calcium
522 Channels in Mouse Taste Cells, *PLoS ONE* 8 (6) (e68174) (2013).

523 [16] M.R. Rebello, K.F. Medler, Ryanodine receptors selectively contribute to the formation of
524 taste-evoked calcium signals in mouse taste cells, *Eur J Neurosci* 32(11) (2010) 1825-35.

525 [17] G. Manning, G.D. Plowman, T. Hunter, S. Sudarsanam, Evolution of protein kinase signaling from
526 yeast to man, *Trends Biochem Sci* 27(10) (2002) 514-520.

527 [18] P. Giansanti, J.R.P.M. Strating, K.A.Y. Defourny, I. Cesonyte, A.M.S. Bottino, H. Post, E.G.
528 Viktorova, V.Q.T. Ho, M.A. Langereis, G.A. Belov, E.N.M. Nolte-t Hoen, A.J.R. Heck, F.J.M. van
529 Kuppeveld, Dynamic remodelling of the human host cell proteome and phosphoproteome upon
530 enterovirus infection, *Nat Commun* 11(1) (2020).

531 [19] S. Prsic, S. Dankwa, D. Schwartz, M.F. Chou, J.W. Locasale, C.M. Kang, G. Bemis, G.M. Church, H.
532 Steen, R.N. Husson, Extensive phosphorylation with overlapping specificity by Mycobacterium
533 tuberculosis serine/threonine protein kinases, *Proceedings of the National Academy of Sciences of*
534 *the United States of America* 107(16) (2010) 7521-7526.

535 [20] S. Xiao, Y.Q. Zhang, P.P. Song, J.B. Xie, G.C. Pang, The investigation of allosteric regulation
536 mechanism of analgesic effect using SD rat taste bud tissue biosensor, *Biosens Bioelectron* 126 (2019)
537 815-823.

538 [21] L.H. Wei, L.X. Qiao, G.C. Pang, J.B. Xie, A kinetic study of bitter taste receptor sensing using
539 immobilized porcine taste bud tissues, *Biosens Bioelectron* 92 (2017) 74-80.

540 [22] Q.D. Xu, D.Q. Lu, G.C. Pang, Comparative study of hGPR120 receptor self-assembled nano-gold
541 sensor and tissue sensor, *Sensor Actuat B-Chem* 320 (2020).

542 [23] H. Lee, L.J. Macpherson, C.A. Parada, C.S. Zuker, N.J.P. Ryba, Rewiring the taste system, *Nature*
543 548(7667) (2017) 330-+.

544 [24] Y.L. Huang, D.Q. Lu, H. Liu, S.Y. Liu, S. Jiang, G.C. Pang, Y. Liu, Preliminary research on the
545 receptor-ligand recognition mechanism of umami by an hT1R1 biosensor, *Food Funct* 10(3) (2019)
546 1280-1287.

547 [25] S.R. Ahn, J.H. An, H.S. Song, J.W. Park, S.H. Lee, J.H. Kim, J. Jang, T.H. Park, Duplex Bioelectronic
548 Tongue for Sensing Umami and Sweet Tastes Based on Human Taste Receptor Nanovesicles, *Acs Nano*
549 10(8) (2016) 7287-7296.

550 [26] J.A. Ubersax, J.E. Ferrell, Mechanisms of specificity in protein phosphorylation, *Nat Rev Mol Cell*
551 Bio 8(7) (2007) 530-541.

552 [27] S. Lim, J. Jung, U. Yunusbaev, R. Ilyasov, H.W. Kwon, Characterization and its implication of a
553 novel taste receptor detecting nutrients in the honey bee, *Apis mellifera*, *Sci Rep-Uk* 9 (2019).

554 [28] D.A. Arvanitis, E. Vafiadaki, D. Sanoudou, E.G. Kranias, Histidine-rich calcium binding protein: The
555 new regulator of sarcoplasmic reticulum calcium cycling, *J Mol Cell Cardiol* 50(1) (2011) 43-49.

556 [29] I. Jardin, J.J. Lopez, A. Berna-Erro, G.M. Salido, J.A. Rosado, Homer Proteins in Ca²⁺ Entry, *Iubmb*
557 Life 65(6) (2013) 497-504.

558 [30] M. Yamamoto, Y. Terada, T. Motoyama, T. Saito, K. Ito, Sweet proteins lysozyme and thaumatin
559 are protein-type agonists for the calcium-sensing receptor, *Biochem Bioph Res Co* 521(1) (2020)
560 227-231.

561 [31] A. Tjondrokoesoemo, N. Li, Z. Pan, C.J. Ferrante, N. Weisleder, J.J. Ma, Inositol 1,4,5
562 Trisphosphate Receptor Type 1 (IP(3)R1) Activate Ryanodine Receptor (RyR1) to Mediate Ca²⁺ Spark
563 Signaling in Adult Mammalian Skeletal Muscle, *Biophys J* 102(3) (2012) 226a-226a.

564 [32] T. Gutierrez, H. Qi, M.C. Yap, N. Tahbaz, L.A. Milburn, E. Lucchinetti, P.H. Lou, M. Zaugg, P.G.
565 LaPointe, P. Mercier, M. Overduin, H. Bischof, S. Burgstaller, R. Malli, K. Ballanyi, J.W. Shuai, T.
566 Simmen, The ER chaperone calnexin controls mitochondrial positioning and respiration, *Sci Signal*
567 13(638) (2020).

568 [33] C. Appenzeller-Herzog, T. Simmen, ER-luminal thiol/selenol-mediated regulation of Ca²⁺
569 signalling, *Biochem Soc T* 44 (2016) 452-459.

570 [34] J. Chandrashekar, C. Kuhn, Y. Oka, D.A. Yarmolinsky, E. Hummler, N.J.P. Ryba, C.S. Zuker, The cells
571 and peripheral representation of sodium taste in mice, *Nature* 464(7286) (2010) 297-301.

572 [35] J.K. Roebber, S.D. Roper, N. Chaudhari, The Role of the Anion in Salt (NaCl) Detection by Mouse
573 Taste Buds, *The Journal of Neuroscience* 39(32) (2019) 6224-6232.

574 [36] S. Lee, V. Augustine, Y. Zhao, H. Ebisu, B. Ho, D. Kong, Y. Oka, Chemosensory modulation of
575 neural circuits for sodium appetite, *Nature* 568(7750) (2019) 93-+.

576 [37] B. Teng, C.E. Wilson, Y.-H. Tu, N.R. Joshi, S.C. Kinnamon, E.R. Liman, Cellular and Neural
577 Responses to Sour Stimuli Require the Proton Channel Otop1, *Curr Biol* 29(21) (2019) 3647-3656.e5.

578 [38] K.L. Mueller, M.A. Hoon, I. Erlenbach, J. Chandrashekar, C.S. Zuker, N.J.P. Ryba, The receptors and
579 coding logic for bitter taste, *Nature* 434(7030) (2005) 225-229.

580 [39] B. Dutta Banik, Martin, Kay, Loney, Nelson, Ahart, Kemp, Kemp, Torregrossa, Medler A subset of
581 broadly responsive Type III taste cells contribute to the detection of bitter, sweet and umami stimuli,
582 *Plos Genet* (2020).

583 [40] Y. Kusuhashi, R. Yoshida, T. Ohkuri, K. Yasumatsu, A. Voigt, S. Hubner, K. Maeda, U. Boehm, W.
584 Meyerhof, Y. Ninomiya, Taste responses in mice lacking taste receptor subunit T1R1, *J Physiol-London*
585 591(7) (2013) 1967-1985.

586 [41] S.D. Roper, N. Chaudhari, Taste buds: cells, signals and synapses, *Nat Rev Neurosci* 18(8) (2017)
587 485-497.

588 [42] W. Feng, J.C. Tu, P. Pouliquin, E. Cabrales, X.H. Shen, A. Dulhunty, P.F. Worley, P.D. Allen, I.N.
589 Pessah, Dynamic regulation of ryanodine receptor type 1 (RyR1) channel activity by Homer 1, *Cell*
590 *Calcium* 43(3) (2008) 307-314.

591 [43] J.M. Eltit, W. Feng, P. Worley, P.D. Allen, J.R. Lopez, I.N. Pessah, Homer-RyR1 Associations are
592 Physiological Regulators of Intracellular [Ca²⁺], *Biophys J* 96(3) (2009) 107a-107a.

593 [44] A.M. Bellinger, S. Reiken, M. Dura, P.W. Murphy, S.X. Deng, D.W. Landry, D. Nieman, S.E. Lehnart,
594 M. Samaru, A. LaCampagne, A.R. Marks, Remodeling of ryanodine receptor complex causes "leaky"
595 channels: A molecular mechanism for decreased exercise capacity, *Proceedings of the National*
596 *Academy of Sciences of the United States of America* 105(6) (2008) 2198-2202.

597 [45] A.J. Pollak, K. Haghighi, S. Kunduri, D.A. Arvanitis, P.A. Bidwell, G.S. Liu, V.P. Singh, D.J. Gonzalez,
598 D. Sanoudou, S.E. Wiley, J.E. Dixon, E.G. Kranias, Phosphorylation of serine96 of histidine-rich calcium-
599 binding protein by the Fam20C kinase functions to prevent cardiac arrhythmia, *Proceedings of the*
600 *National Academy of Sciences of the United States of America* 114(34) (2017) 9098-9103.

601 [46] Q.B. Jiao, H. Takeshima, Y. Ishikawa, S. Minamisawa, Sarcoplasmic reticulum plays a critical role in
602 age-related cardiac dysfunction due to decreases in SERCA2a expression and activity, *Cell Calcium*
603 51(1) (2012) 31-39.

604 [47] M. Salanova, P. Volpe, D. Blottner, Homer protein family regulation in skeletal muscle and
605 neuromuscular adaptation, *lubmb Life* 65(9) (2013) 769-776.

606 [48] J.C. Tu, B. Xiao, J.P. Yuan, A.A. Lanahan, K. Leoffert, M. Li, D.J. Linden, P.F. Worley, Homer binds a
607 novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors,
608 *Neuron* 21(4) (1998) 717-726.

609 [49] A. Mizutani, Y. Kuroda, A. Futatsugi, T. Furuichi, K. Mikoshiba, Phosphorylation of Homer3 by
610 calcium/calmodulin-dependent kinase II regulates a coupling state of its target molecules in Purkinje
611 cells, *J Neurosci* 28(20) (2008) 5369-5382.

612 [50] N.E. Clifton, S. Trent, K.L. Thomas, J. Hall, Regulation and Function of Activity-Dependent Homer
613 in Synaptic Plasticity, *Molecular Neuropsychiatry* 5(3) (2019).

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Table 1 The K_a values of sweet and umami molecules based on rat taste bud tissue sensor

Taste substances	Kinetic equation	R^2	Double reciprocal equation	R^2	K value	n
MSG	$\Delta I = 9.0692C * 10^{-14} / (1.73219 + C * 10^{-13})$	0.980	$1/\Delta I = 1.12966 + 1.63168 * 10^{-13} 1/C$	0.970	1.4444E-13	2.889
IMP	$\Delta I = 6.5478C * 10^{-15} / (3.06877 + C * 10^{-14})$	0.957	$1/\Delta I = 1.61363 + 3.08669 * 10^{-14} 1/C$	0.954	1.9129E-14	3.826
GMP	$\Delta I = 7.8137C * 10^{-15} / (1.51216 + C * 10^{-14})$	0.983	$1/\Delta I = 1.30753 + 1.63601 * 10^{-14} 1/C$	0.981	1.25122E-14	2.502
SUC	$\Delta I = 7.5621C * 10^{-14} / (1.45738 + C * 10^{-13})$	0.961	$1/\Delta I = 0.67719 + 2.01737 * 10^{-14} 1/C$	0.981	2.97903E-14	5.958
Aspartame	$\Delta I = 6.9162 * 10^{-13} / (0.09824 + C * 10^{-12})$	0.983	$1/\Delta I = 1.44687 + 1.411 * 10^{-13} 1/C$	0.951	1.15078E-14	2.302
Sucralose	$\Delta I = 7.109 * 10^{-13} / (0.13136 + C * 10^{-12})$	0.975	$1/\Delta I = 1.40867 + 1.87566 * 10^{-13} 1/C$	0.989	1.44267E-14	2.885
Thaumatococcus	$\Delta I = 3.2181 * 10^{-13} / (0.23347 + C * 10^{-12})$	0.983	$1/\Delta I = 3.17126 + 6.8347 * 10^{-13} 1/C$	0.997	2.07885E-14	4.158
Sucrose	$\Delta I = 4.14 * 10^{-13} / (0.09687 + C * 10^{-12})$	0.996	$1/\Delta I = 2.425427 + 2.3906 * 10^{-13} 1/C$	0.962	0.904972E-14	1.810

Figure caption

Fig. 1 The schematic diagram of approach to protein phosphorylation analysis in rat bud tissue after sweet and umami stimuli

Fig. 2 a: The information of differential genes in the rat bud tissues after 8 taste stimuli treatment including sucrose, sucralose, aspartame, thaumatococcus, succinic acid disodium salt (SUC), monosodium glutamate (MSG), guanosine 5'-monophosphate disodium salt (GMP) and disodium Inosinate (IMP); b: Venn diagram showing the distribution of sweet-related phosphoproteins in rat bud tissues; c: Venn diagram showing the distribution of umami-related phosphoproteins in rat bud tissues; d: Venn diagram showing 8 common differential proteins in all treated bud tissues; e: Top enriched pathways in 8 common proteins in the treated bud tissues; f: The related protein to RYR1 in taste bud tissue association predicted network by STRING

(<http://string-db.org>).

Fig. 3 Volcano plots of protein samples from rat bud tissue treated with various sweet and umami substances. Compared to black sample, Fold changes in proteins abundance (Proteins passing both cutoffs) for each treated sample are shown as a function of statistical significance. Significance cutoffs were $p\text{-value} \leq 0.05$, $FC \geq 1.2$ and $FC \leq 0.83$. Each protein is represented with a single data point, corresponding to the peptide with the lowest p-value. The common interactor of the bud tissues treated with 8 taste substances were highlighted in cyan and labeled with their gene names. a: Sucrose; b: Sucralose; c: Aspartame; d: Thaumatin; e: GMP; f: IMP; g: MSG; h: WSA

Fig. 4 The confirmation of RYR1 roles in sweet and umami perception. a: The response of the taste bud tissue after ryanodine treatment to MSG solution (10^{-12} ~ 10^{-6} mol/L) based on rat taste bud tissue sensor; b: The influence of RYR1 on taste perception based on behavioral tests (water intake volume); c: Expression profiling of fluorescently labelled RYR1 protein; d: Expression profiling of sweet and umami receptor (T1R1, T1R2 and T1R3) in two rat taste bud tissue; e: Confocal microscopy images of immunostaining illustrating the expression of RYR1 (green) and calnexin (red) in bud tissue and skeletal muscle.

Fig. 5 Model proposing the phosphorylation of HRC and RYR1-mediated sweet and umami signaling in metabolic cycles. After sweet and umami substances intake (Full state), RYR1 channel is closed in virtue of its dephosphorylation, and opening SERCA pump is regulated by HRC-p to increase Ca^{2+} flux from the cytosol to the ER. The digestion period of nutrient, RYR1 channel is opened by its phosphorylation, accompanied by Ca^{2+} flux from the ER to the cytosol and the dephosphorylation of

HRC. The body goes hungry state until HRC in the ER was fully dephosphorylated, and SERCA pump is closed. SERCA pump is opened again by HRC-p to increase Ca^{2+} flux from the cytosol to the ER, and RYR1 channel is closed again since food intake beginning.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5